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CONTENTS

PEARSE, A. S. *The Growth of the Painted Turtle* 145

BODINE, JOSEPH H. *A Note on the Toxicity of Acids for Mosquito Larvæ* 149

GALTSOFF, PAUL S. *The Amœboid Movement of Dissociated Sponge Cells* 153

HEGNER, ROBERT W. *Observations and Experiments on Euglenoidina in the Digestive Tract of Frog and Toad Tadpoles* 162

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BIOLOGICAL BULLETIN

THE GROWTH OF THE PAINTED TURTLE.

A. S. PEARSE,

ZOÖLOGICAL LABORATORY, UNIVERSITY OF WISCONSIN.

As Agassiz (1857) pointed out, the growth of turtles is exceedingly slow. After comparing turtles of different sizes, Agassiz concluded that the eastern painted turtle (*Chrysemys picta*), after the eggs from which it hatched were laid, attained about the following lengths after one year: 26.5 mm.; 2:42; 3:51; 4:54; 5:59; 6:66; 7:72.5; 8:74; 9:77; 10:80; 13:92; 24:121. He affirms that this turtle does not lay eggs until it has attained an age of ten to eleven years. Lucas (1922) states that there are authentic records of tortoises that have lived to be one hundred and fifty years old. Barney (1922) has given a very careful account of the growth and breeding of the diamond-back terrapin when reared under cultural conditions in pens. He found that when domestic terrapins are fed during the winter, egg production occurs as early as the fourth year of age, but usually begins in the fifth or sixth year. Terrapins may reach a length of 130 mm. to 150 mm. in four years when fed in winter, and in six to seven years when allowed to hibernate. The maximum growth in length recorded for one year was 81 mm., and for two years 104 mm.

Since 1919 the writer has had opportunity to study the growth of the western painted turtle.[1] At various times, 406 turtles were marked with aluminum tags and immediately released in University Bay, Lake Mendota. These were measured when they were released and were caught at intervals and measured again. In this way the rate of growth was determined. The portion of the bay behind the bar, where the turtles were studied, is nowhere more than 1.5 meters in depth, has a soft, muddy

[1] Ruthven's (1912) *Chrysemys belli* Gray and *C. cinera* (Bonnaterre) appear to intergrade in this locality, but a majority of the individuals resemble the former.

TABLE I.

LIST OF RECORDS OF GROWTH OF TURTLES.

Abbreviations refer to months, from January to December, in the following order: J, F, M, Ap, My, Ju, Jl, Au, S, O, N, D, and indicate when each turtle was tagged and released.

Time Elapsed.			Length.			Gain.
Years.	Months.	Days.	Begin.		End.	
0	11	18	Jl	40.0	62.5	22.5
0	0	17	Jl	42.5	51.0	7.5
0	0	16	Jl	47.5	52.0	4.5
1	9	22	S	51	84.0	33.0
1	9	29	S	51	85.0	34.0
0	0	19	Jl	54	58.0	4.0
0	1	4	Jl	54	62.2	8.2
0	11	18	Jl	54	73.3	19.7
2	2	9	Jl	54	84.0	30.0
0	11	20	Jl	58.0	77.5	19.5
1	9	15	S	59	77.0	18.0
1	11	6	S	59	83.0	24.0
0	1	11	Ju	60	68.3	8.3
0	0	27	Jl	60	61.0	1.0
0	0	5	Jl	60	60.5	0.5
0	0	7	Jl	60	61.0	1.0
0	2	11	Jl	60	73.3	13.3
1	2	4	Jl	62	87.5	25.5
0	11	18	Jl	64	82.0	18.0
0	11	18	Jl	65	78.0	13.0
0	1	4	Jl	65	73.0	8.0
2	11	14	S	67	94.4	27.4
0	1	4	Jl	68	71.3	3.3
1	0	2	Ju	69	83.2	16.2
0	11	11	Jl	70.5	90.8	20.3
1	1	26	Jl	70.5	97.0	26.5
1	1	16	Jl	74	85.0	11.0
0	0	19	Jl	77	80.5	3.5
0	0	23	Jl	77	83.0	6.0
1	2	5	Jl	77	96.0	19.0
0	11	17	Jl	78.0	98.0	20.0
1	2	10	Jl	78	98.0	20.0
0	11	13	Jl	81.0	89.7	8.7
3	11	19	Au	82.0	95.8	13.8
1	5	16	F	87.0	91.0	4.0
0	2	18	Jl	89.5	91.0	1.5
0	11	17	Jl	89.5	92.3	2.8
1	2	21	Ju	90.0	108.2	18.2
0	3	3	Ju	90	94.5	4.5
0	7	14	My	91.0	92.0	1.0
1	2	4	My	91.0	106.0	15.0
1	9	27	S	95.0	97.3	2.3
0	1	3	Ju	97.0	98.0	1.0
1	0	3	Ju	97.0	99.6	2.6
1	2	0	My	99.0	101.5	2.5
1	0	6	Ju	99.0	102.0	3.0
1	1	5	My	100.0	103.5	3.5

Table I, *Continued*

Time Elapsed.			Length.		Gain.
Years.	Months.	Days.	Begin.	End.	
0	11	23	Jl 101.0	110.2	9.8
1	0	2	Ju 101.0	111.0	10.0
1	2	4	My 103.0	106.7	3.7
2	2	10	My 103.0	109.7	6.7
2	11	22	S 103.0	113.5	10.5
0	1	0	Jl 103.0	103.0	0.0
0	10	18	Ju 104.0	107.0	3.0
1	11	27	S 105.0	108.9	3.9
2	0	6	S 105.0	109.5	4.5
1	2	11	My 105.0	110.0	5.0
1	2	0	Jl 105.0	110.0	5.0
1	1	25	My 108.5	111.0	2.5
1	9	20	S 112.0	119.0	7.0
1	2	7	My 112.0	112.2	0.2
1	1	5	My 112.0	114.0	2.0
0	11	18	Jl 113.0	123.2	10.2
0	11	14	Jl 113.0	115.0	2.0
1	2	11	My 114.0	118.5	4.5
1	2	4	My 116.0	117.3	1.3
1	0	6	My 118.0	120.0	2.0
1	2	4	My 120.0	123.0	3.0
0	11	10	Jl 121.0	128.7	7.7
1	1	22	My 121.5	123.0	1.5
1	2	4	Jl 127.0	130.5	3.5
0	9	0	Ju 127.3	133.5	6.2
0	0	16	My 129.0	129.0	0.0
2	1	4	Au 130.0	137.0	7.0
4	0	6	Au 130.0	139.0	9.0
2	1	4	S 130.0	136.0	6.0
0	2	11	Jl 131.0	131.5	0.5
0	9	10	Jl 131.0	131.5	0.5
1	1	27	My 132.0	132.0	0.0
2	2	4	My 132.0	135.5	3.5
1	2	6	My 134.0	139.0	5.0
0	1	1	Jl 140.0	140.0	0.0
2	2	3	Jl 140.0	144.0	4.0
1	2	26	Ju 140.0	143.3	3.5
1	2	0	My 142.0	144.0	2.0
1	1	20	My 142.0	142.0	0.0
0	11	13	Jl 148.5	150.0	1.5

bottom, and maintains a vigorous growth of aquatic plants. An abundance of food was therefore available.

The records are summarized in Tables I. and II. It will be noted that some individuals grew very rapidly during a few days, and that other individuals of about the same size increased little or not at all during a considerable period of time. Such differences are probably correlated with the shedding of the dermal plates of the shell, growth being rather rapid immediately after the plates are lost.

TABLE II.

GROWTH OF TURTLES OF DIFFERENT LENGTHS.

Length in *mm.*	Number of records.	Average Rate of Growth; *mm.* Per Year.	Estimated Average Weight; Grams.	Estimated Average Weight Increase; Grams Per Year.	Percentage of Increase.
40– 50	2	32.7	19	13.8	73
50– 60	10	17.0	36	11.1	31
60– 70	12	16.7	50	12.7	25
70– 80	8	19.0	68	15.9	25
80– 90	3	4.2	106	5.2	5
90–100	11	6.0	134	8.5	6
100–110	13	3.5	170	5.7	3.3
110–120	8	3.1	233	6.3	2.7
120–130	6	4.2	243	8.2	3.4
130–140	8	1.5	310	3.4	2.4
140–150	6	1.6	362	3.9	2.7

Table II. shows that a turtle nearly doubles its length and weight during the second year of its life. After twelve years it would be about 135 mm. long and the growth rate would have decreased to about one thirtieth of that during the first two years. An ordinary adult turtle measuring 150 mm. in length is, using the data here presented as a basis for computation, about twenty-five years of age (since the eggs from which it hatched were laid). The largest turtle measured from Lake Mendota was 170 mm. in length. It was perhaps fifty years of age.

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A NOTE ON THE TOXICITY OF ACIDS FOR MOSQUITO LARVÆ.

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Interest in the resistance of mosquito larvæ to various physical and chemical reagents has been largely, if not solely, directed toward the discovery of practical methods for the eradication of the mosquito. A large amount of literature dealing with this phase of the subject is now available. The effects of various solutions of salts on mosquito larvae have also been rather extensively investigated and it has been found that the animals are able to withstand rather high concentrations of pure salt solutions (Mac Fie (1), Chidester (2), Sen (3), Sharma (4), etc.). It has recently been pointed out by MacGregor (5) that mosquito larvæ are able to live and develop in extremely high concentrations of acid, *e.g.*, acid of $P_H = 4.4$. In view of these last mentioned observations, it was thought advisable to test the toxicity of a series of acids of different concentrations for mosquito larvæ and the present paper embodies results obtained from such experiments.

The larvæ used in all experiments, *Culex pipiens*, were obtained in large numbers from small, stagnant pools usually found in uncovered containers. The entire culture as found was brought into the laboratory and tests carried out at the same time and with the same group of animals, all of which had presumably been under identical conditions. Both young and old larvæ were used and differences due to age noted. The animals were removed from the cultures by means of a wide-mouth pipette and transferred to a syracuse watch glass with as little of the culture medium as possible. In this way a large number of larvæ could easily be obtained for use in each experiment. Fifty to sixty animals were used in testing the effect of any concentration of reagents. The chemicals used (10 c.c.) were put into covered syracuse watch glasses; the larvæ were quickly injected into the solution and then observed until dead under a binocular micro-

scope. The fatal exposure was taken at that time when approximately one half of the larvæ were killed, *i.e.*, when movements of the heart and alimentary canal ceased. Many cultures were used in experiments and slight variations in their resistance were shown but in the following only average results will be given. It is, however, of some interest to note that in practically all the cultures from which larvæ were obtained the hydrogen ion concentration showed them to be neutral or slightly alkaline in reaction (P_H = 7.0–7.4). The chemicals used were, hydrochloric, acetic, oxalic, butyric, salicyclic and carbonic acids and mercuric chloride.

FATAL EXPOSURES IN MINUTES TO DIFFERENT STRENGTHS OF ACIDS (TEMP. 25° C.).

Normality of Acid.	HCl.	Oxalic.	Salicyclic.	Butyric.	Acetic.
0.5	9.5	.....	.....	.....	.....
0.2	42.0	.....	.....	.....	.....
0.1	74.0	39.0	.....	72.0	191.0
0.01	293.0	52.0	48.0	1440.0	1440.0+
0.001	1440.0+	1440.0	1200.0	1440.0+	1440.0++
0.0001	.....	1440.0+	1440.0+	.....	.....

From the above table showing the length of life in minutes of larvæ in various strengths of acids it is evident that the animals are able to withstand abnormally high concentrations of acids for rather long periods of time. This remarkable resistance of mosquito larvæ is more strikingly shown when compared with that found for other forms—*e.g.*, Honda (6) found that the free-living nematode *Rhabditis elegans* withstood 0.01 normal HCl for 60 minutes, *Daphnia* for 23 minutes, tadpoles for 12 minutes and paramecium for 1 minute. (Personal communication to be published in *Journal of Experimental Zoölogy*.) MacArthur (7) found that Planarians are killed in a very short time by exposure to HCl of P_H 2–4.5. It has also been found by the author (8) that cysts of *Colpoda* withstand 0.001 N HCl for a strikingly long time.

That the hydrogen ion concentration is not necessarily the only factor in the toxicity of acids for larvæ is shown by comparing the effects of a saturated solution of CO_2 in H_2O of a P_H of approximately 3.7 with a solution of HCl of the same P_H value.

Larvæ in the CO_2 solution become motionless almost at once and the movements of heart and alimentary canal also quickly cease, while in HCl of the same P_H value they are apparently unaffected for over 24 hours. The more rapid penetration rate and mode of action of CO_2 as pointed out by Jacobs (9), doubtless account for the differences observed in the effect of the two reagents.

It is also of much interest to know in what manner the acids kill the animals, whether they enter the chitinous covering or enter by the mouth or anus through the alimentary canal. By using pupae, which are known not to eat nor to have external openings as in the larvæ, it is found that the acids do not kill them for many hours, considerably in excess of the lethal exposure for larvæ. From this fact it seems reasonable to assume that the larvæ are killed by the entrance of the reagent orally rather than cutaneously. The present discussion, however, deals primarily with the resistance of the animals to the reagents rather than with their mode of killing. Younger and smaller larvæ, and these doubtless have thinner chitin, are killed somewhat more quickly than older individuals.

The general order of toxicity of the acids used for the larvæ is, salicyclic > oxalic > HCl > butyric > acetic. This series is strikingly similar to that found by Haas (10) for plants, by Collett (11) for protozoa and by Bodine (8) for cysts of *Colpoda*.

FATAL EXPOSURES IN MINUTES TO DIFFERENT PERCENTAGES OF $HgCl_2$ (TEMP. 25° C.).

Per Cent. $HgCl_2$.	Time in Minutes.
0.05	305.0
0.10	155.0
0.50	48.3
1.00	26.5
2.00	20.0

Mercuric chloride in various concentrations was used and here again the resistance of the larvæ is of considerable interest. The above table shows the effect of different percentages of this salt. Honda (6) found that the free-living Nematode *Rhabditis elegans* withstood 0.05 per cent. $HgCl_2$ for 60 minutes; *Daphnia*, 25 minutes and tadpoles, 5 minutes. Mosquito larvæ are about 5 times as resistant to $HgCl_2$ as the most resistant form used by this author. Sen (3), with other species of mosquito larvæ (*Ste-*

gomyia albopicta) found that the animals were killed at once in a 0.1 per cent. $HgCl_2$ solution. This difference in length of time of fatal exposure to this concentration from the present result is doubtless due to the differences in resistance of the two species as well as to the end point taken in the experiments. Sen evidently used cessation of body movement while in the present investigation cessation of movements of heart and alimentary canal were taken as the end point.

SUMMARY.

1. Mosquito larvæ (*Culex pipiens*) were found to be extremely resistant to rather high concentrations of various acids.

2. The order of toxicity of the acids used is, salicylic > oxalic > HgCl > butyric > acetic.

3. The chemicals seem to penetrate the animal orally and not cutaneously.

4. Animals withstand rather high concentration of $HgCl_2$, considerably in excess of that found for other organisms cited.

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THE AMŒBOID MOVEMENT OF DISSOCIATED SPONGE CELLS.[1]

PAUL S. GALTSOFF.

INTRODUCTION.

In 1907 H. V. Wilson discovered a very interesting case of regeneration in siliceous sponges from dissociated tissue cells. The same phenomenon was observed in Hydroids, *Alcyonaria.* and Asteriæ (Wilson, 1911), in fresh water sponges (K. Müller, 1911) and in calcareous sponges (Huxley, 1920).

Twelve years before Wilson's discovery Roux (1895) described a similar phenomenon. He found that the blastomeres of the frog egg, artificially separated at an early stage of segmentation, and placed in water a short distance apart, slowly approached one another until they came into contact. Roux called this phenomenon cytotropism to correspond with other tropisms known in the scientific literature. He regarded cytotropism as a special case of chemotropism. This opinion was based on theoretical considerations as no experiments were made to prove it.

The term cytotropism although not definitely accepted has been used in various text books and scientific papers. Apparently the sponges with their ability to form the conglomerates from the dissociated cells afford the best opportunity for a study of this phenomenon.

Wilson and Müller (l.c.) made their studies on the regeneration of sponges after dissociation only after the aggregates began to form. Prior to this they made no exhaustive study, and merely stated that the separated cells coalesce and form aggregates. They did not study the amœboid movement which leads to the coalescence of cells and which is the main purpose of the present investigation. The work was started in 1920 at the Marine Biological Station at Sebastopol (Black Sea) and continued in 1921 at Woods Hole and in the Zoölogical Laboratory of Columbia University.

[1] Contributions from the Sebastopol Biological Station, Crimea, and the Marine Biological Laboratory, Woods Hole, Mass.

The writer desires to express his gratitude to Dr. F. R. Lillie, Director of the Marine Biological Laboratory at Woods Hole, for accomodations there and to Dr. T. H. Morgan, of the Department of Zoölogy, Columbia University, for the courtesy of extending to him laboratory privileges.

Species Used for Experiments.

The coalescence of cells after their dissociation has been observed in many species of sponges. It is probable that all sponges possess this ability, but the formation of dense conglomerates and the power to regenerate a new organism occur only in a few forms. The species tested by the writer are as follows: Black Sea sponges—*Halichondria grossa* Schm., *Petrosia clavata* B. Cor., *Reniera densa* Bowerb., *Reniera informis* Schw., *Esperella lorenzii* Sch., *Kowalevskiella gracilis* Swarc., *Spongelia* sp., *Sycon* sp.; Woods Hole sponges—*Microciona prolifera* Verr., *Cliona celata* Gr., and *Grantia* sp.

Upon dissociation the cells of these species are able to coalesce and to form aggregates, but the grade of the formation varies. The best forms are *Reniera informis*, *Reniera densa*, *Petrosia coriacea*, and *Microciona prolifera*. The formation of aggregates in these species requires less time, the aggregates are more strongly attached to the substratum and they quickly transform themselves into new sponges.

Unfortunately many of the microphotographs, drawings, and other data collected by the writer at Sebastopol were lost owing to unavoidable circumstances and therefore the present work deals chiefly with the experiments made on *Microciona prolifera* at Woods Hole.

Amœboid Movement of Dissociated Cells.

The suspension of dissociated *Microciona* cells, obtained by squeezing the sponge through bolting silk No. 20, consists of three classes of cells, each of which can be easily recognized. The most abundant are the archaeocytes, nonspecialized, reddish cells about 8 microns in diameter and loaded with granules. Two kinds of these cells can be discriminated: the endoplasm of the first contains red pigment granules, to which the red color of the sponge is mainly due; the second contains in addition to a less

abundant red pigment many dark yellow-greenish granules. Both forms are able to put out rounded or sometimes elongated pseudopodia and to display an active amœboid movement.

The second class consists of spheroidal dermal cells differing in size from 8 to 3 microns in diameter. They move very slowly, putting out short rounded pseudopodia.

To the third class belong the collar cells or so called choanocytes. They are partially dedifferentiated having lost their collars but still possess a long slender flagellum which continues to vibrate for at least three hours.

The process of reunion of the dissociated elements of the sponge tissue consists of following stages; sinking of the cells and adhesion with one another, adhesion to the bottom, amœboid movement and coalescence of cells, movement and coalescence of aggregates. The process of the formation of the aggregates can be easily observed in a microaquarium or in a hanging drop on a hollow slide. In both cases the globular aggregates are formed within three to four hours, the difference being that in the microaquarium the aggregates strongly adhere to the glass, while in a hanging drop they remain floating.

Among the different tissue elements of sponge the archaeocytes are the most active; some of them may send out pseudopods within a few minutes after the sponge was squeezed. They begin to move as soon as they come in contact with the bottom. The coalescence may even occur while the cells are still in suspension.

The character of the amœboid movement of both yellow and red archæocytes is the same; the cells put out large hyaline pseudopods and creep in different directions. The inner granuloplasm of the cells appears to be more viscous than their surface hyaline layer. This can be easily observed when two cells come in contact with one another. When two archæocytes touch one another their external hyaloplasm spreads out from both sides of the line of contact and flows round their bodies. Sometimes one can notice how the hyaloplasm is pressed away from the contact line between the cells and surrounds their bodies.

Often the archæocytes lose small drops of hyaloplasm which remain behind them indicating the route of the cell. Another archæocyte passing the same way may wipe out these drops which coalesce with its protoplasm.

On coalescence the inner granular protoplasm of the archæocytes remains unmixed; the granuloplasm of various cells occupies a definite portion of the aggregate, while the hyaloplasm forms a common mass surrounding the whole group.

By pressing the coverglass under which the aggregates are lying or by violently shaking a dish containing aggregates the cells can be separated after which they are able to coalesce again.

The amœboid movement of the archæocytes is irregular; some of them move actively while the others remain motionless or creep very slowly over the bottom. There is no visible difference between sluggish and active archæocytes. Frequently after being immobile for 60 or 80 minutes the archæocyte becomes active and starts to move rapidly.

In many cases when two archæocytes coalesce the aggregate remains motionless for a few minutes after which it starts to move in another direction. The coalescence with a small dermal cell or with a choanocyte does not disturb its movement; it continues to move as if there was nothing in its route.

The small dermal cells move very slowly or not at all. The choanocytes do not display an amœboid movement but are able to displace themselves by means of their active flagella.

The ectoplasmic layer of the isolated archæocytes is fluid and sticky. The cells easily adhere to different objects which come in contact with them. The adhesiveness of their protoplasm can be strikingly seen in a suspension containing starch grains. Each aggregate formed in such suspension is surrounded with a ring of starch grains. This occurs because the starch grains adhere strongly to the cells; as the aggregates move and turn in different directions they become surrounded with grains with which they accidentally come in contact. When moving the cells are able to push along or to carry various foreign bodies which they meet in their route. In one observation a small aggregate was found strong enough to push a group of five starch grains each of which was larger than the aggregate itself.

The formation of the aggregate from dissociated tissue cells is due to the motility of certain archæocytes and to the stickiness of their outer layer of protoplasm. The various cells are uniformly distributed in the suspension and over the bottom of the dish; each active archæocyte moves back and forth over a definite

area and therefore cleans up the corresponding portion of the bottom, collects all cells lying in its route and finally forms an aggregate.

A well formed 24-hour-old aggregate of *Microciona* has the form of a ball; its surface is smooth and a thin hyaline membrane can be noticed on its periphery. Under unfavorable conditions the aggregates are irregular in form and fail to form a membrane. They are then unable to undergo futrher transformation and to regenerate into a new sponge.

Direction of Cell Movement.

There arises a question whether the approaching and the coalescence of the cells are due to a special kind of chemotropism or "cytotropism" or whether their movement is chaotic and their approach is a matter of a pure accident. If there be a directive force one may expect that the cells will move towards several others which form the centers of the attraction. In other words one ought to be able to detect a definite directive movement.

The simplest way to study the direction of cell movement is to draw contours of cells at definite intervals and then project all the outlines on one surface and in this way to reconstruct the paths of the cells. Such an investigation was made with a camera lucida and a combination of the Zeiss objective E and eyepiece 6 (× 625). A microaquarium was filled with a dilute suspension of Microciona (1 gram of sponge per 200 c.cm. of sea water); a sufficient number of paper sheets were placed on the table at the level of the microscope stage and were pierced at two points. The holes made by the punctures enabled one to put the sheets exactly upon one another after they had been removed from the table. Special precautions were made not to disturb the lower sheets when the upper one was removed. The sketches were made every two minutes and often every minute. The observations lasted from 40 to 190 minutes. After this period the movements are so slow that the continuance of the observation was unpracticable. The temperature of the water during the observations varied from 19° to 21° C. The observations were repeated many times with different colonies of *Microciona;* in all cases the character of the movement was the same, the dif-

ference being only in the velocity and in the duration of the movement.

The examination of the paths of various cells shows that their movement is not at all directed towards one another or towards the group of cells. No one cell could be found which acts as a center of attraction for other cells. The coalescence occurs only when one moving cell happens to touch another one, and to stick to its outer layer. The path of the archæocyte is an irregular winding line; a typical case is shown in Fig. 1. The movement of this archæocyte was followed for 168 minutes. The points where coalescence with other cells occurred are indicated by crosses; the arrows show the direction of the movement. Other cells in the same field of view moved very slowly and passed only few microns; the aggregate was formed exclusively through the activity of the archæocyte.

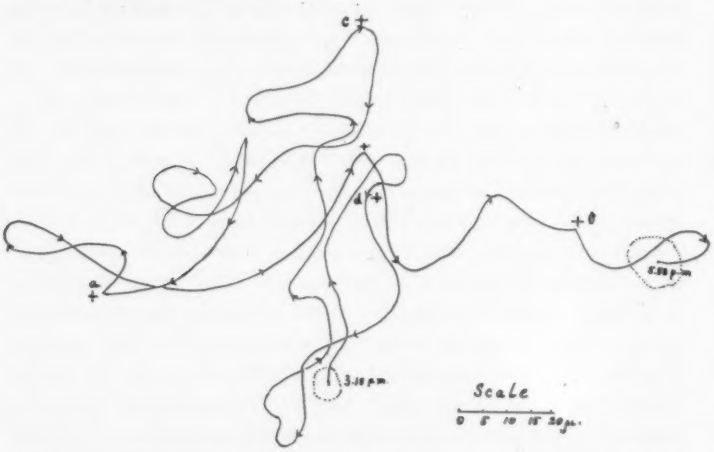

FIG. 1. The path of the archæocyte of *Microciona*; the line representing the movement of the center of the cell. The observation was made with camera lucida, Zeiss obj. E, and eyepiece 6. The outlines were drawn each two minutes.

This archæocyte travelled back and forth in different directions, approaching certain cells from which it withdrew. It coalesced first with the small dermal cell *d*, with the chaonocyte *c*, and with two archæocytes *a* and *b* all of which happened to lie in its route.

The velocity of the movement of the archæocyte is subject to

many fluctuations. Even a fast moving cell sometimes stops and after a short period of rest resumes its movement. The retardation as a rule takes place when the cell changes its direction. The average velocity of an archæocyte measured in ten different experiments varied from .6 to 3.5 microns per one minute; the maximum velocity was as great as 20.0 microns per one minute. This maximum velocity was observed only twice. The distance travelled varied from 64 to 185 microns and active movement lasted from 40 to 168 minutes.

The Behavior of the Dissociated Cells in a Compound Suspension.

The cells of two different species of sponges mixed together coalesce only with cells of their own species. This ability of cells to discern the more foreign elements can be easily observed when two sponges of different colors are used. Wilson (1910) pointed out that the cells of *Microciona* mixed with those of *Lyssodendrix* and *Stylotella* form different clumps each apparently composed of the cells of same species; the clumps could be recognized by their natural colors. The same was found by the writer when a mixed suspension of *Reniera informis*, violet, Reniera densa, gray, and of *Microciona*, red, and *Cliona*, yellow, were tested. The aggregates formed in such emulsions were a violet, gray, red and yellow.

It is quite easy to distinguish the colors of the aggregates but as the color of a single cell is very slight it is impossible to distinguish the species while the cells are in suspension. In order to find how complete is the separation of the cells in a mixed suspension, one of the sponges before the experiment was fed with carmine, the other, with Chinese ink. The red and black granules ingested by the cells serve as definite marks indicating the species.

The suspension contained the cells of *Reniera informis* fed with carmine and that of *Reniera densa* fed with Chinese ink. The resulting aggregates lying close together consist exclusively either of carmine or of ink laden cells. The same occurs in a mixture of *Microciona prolifera* and *Cliona celata*.

Coalescence with the cells of another species never occurs even when the cells are artificially pressed together by centrifuging a

mixed suspension. Examining after 24 hours such a clump of *Microciona* and *Cliona* one can find that in a common yellow mass of Cliona cells the Microciona had formed globular aggregates with marked membranes separating them from cells of the other species.

The formation of the aggregate in a mixed suspension does not go so completely as in a pure one; the aggregates are smaller and correspondingly more numerous than in the control dish. They are unable to undergo further transformation.

The coalescence of the dissociated tissue cells of sponges is apparently the same phenomenon as occurs in the extravasated blood cells of Arthropoda (Tait, 1918, Leo Loeb, 1920). In both cases the mechanical or chemical changes in the environment of the cells lead to its amœboid activity and to formation of aggregates of separated cells. In sponges the archæocytes *i.e.*, non-specialized elements, form aggregates which are able to regenerate a new organism. In blood cells the process does not go so far; the amœbocytes join in clumps and under favorable conditions can form a certain kind of tissue (L. Loeb). There is no indication of chemotropic or cytotropic stimuli in both cases and no such hypothesis is required to explain the results.

Conclusions.

The coalescence of separated sponge cells is the result of two factors: first, amœboid activity of the archæocytes, second a specific physical property of cell protoplasm which enables the cells to coalesce when they come into contact.

The coalescence of cells of two different species never occurs apparently because the physical properties of the protoplasm of the various species are different.

So-called cytotropism or a special kind of chemotropism does not exist in the cases studied.

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OBSERVATIONS AND EXPERIMENTS ON EUGLENOIDINA IN THE DIGESTIVE TRACT OF FROG AND TOAD TADPOLES.

ROBERT W. HEGNER.[1]

On a number of occasions during the past decade the writer has observed living flagellates of the *Euglena* type in the intestinal and rectal contents of frog tadpoles. They were always considered merely accidental inhabitants that had been ingested with the food of the tadpoles and were either immune to the digestive juices and were on their way through the intestine or had not yet succumbed to digestion. The observations and experiments described below, however, furnish evidence (1) that these flagellates are of widespread occurrence among the tadpoles of a number of species of frogs and toads, (2) that they are normal inhabitants of the intestine and rectum of tadpoles in the same sense that the better known protozoa, such as *Opalina*, are, (3) that they persist in starved tadpoles for many days, even after *Opalina* has disappeared, (4) that they retain their green color for a considerable period within the body of the tadpoles, (5) that they can be transferred in the trophozoite stage from one species of tadpole to another with food material, (6) that they differ in structure from any free-living or parasitic Euglenoidina heretofore described, (7) that they do not grow and multiply easily under ordinary culture conditions, (8) that certain free-living species of Euglenoidina are digested by tadpoles that do not digest the entozoic species, and (9) that certain tadpoles that were heavily infected with Euglenoidina did not become full-grown and undergo metamorphosis.

1. *A Comparison of Normal Tadpoles of Rana pipiens with Tadpoles Containing Large Numbers of Species A.*—The first series of observations, recorded in Table I. indicate that the

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euglenoid flagellate that I shall call Species A*, is a constant inhabitant of the tadpoles of *Rana pipiens* from Ithaca, N. Y., and that it may be a factor in the failure of certain specimens to grow and undergo metamorphosis normally. So far as I have been able to learn, this species has not been described although Alexeieff (1912) noted what may have been specimens of this form in the intestines of tadpoles. It is cylindrical and elongate with blunt anterior and posterior ends and decidedly metabolic. It ranges in length from 35 μ to 45 μ and in breadth from 4.8 μ to 6.4μ. The average length of ten specimens was 39.7μ and average breadth, 5.3 μ. There are three flagella almost as long as the body and about 50 green chromatophores, oval or circular in outline, lying in a single layer near the surface of the body. The nucleus is spherical and situated near the center of the body. Near the anterior end is a large spherical reservoir opening to the outside through a cytopharynx. At one side of the reservoir is the red stigma. Figs. 1 to 3 are of three specimens drawn with the camera lucida at a magnification of 1600 diameters. The specimen in Fig. 1 was living when drawn, in Fig. 2, killed and stained with iodine, and in Fig. 3, fixed in Schaudinn's fluid and stained by the iron-hæmotoxylin method.

The tadpoles were collected in artificial ponds at the fish hatchery of Cornell University at Ithaca, N. Y. My attention was directed to their study by Dr. G. C. Embody, who had noted the small size of specimens in one pond as compared with those in a neighboring pond only six feet distant. These ponds were about 4 feet square and 18 inches deep. One was well filled with algæ and the other was almost free from vegetation. Egg masses of *Rana pipiens* had been placed in these two ponds by Dr. Embody at approximately the same time, about April 20. The tadploes were collected seven weeks later (June 8). In Tables I. and II. are presented data regarding the differences (1) between the two sets of tadpoles and (2) between their rectal contents. The following points may be noted.

1. *Size.*—(Table I.) Although the two groups of tadpoles

*After this paper was written I learned that Dr. D. H. Wenrich had also spent the summer of 1922 studying this organism. His work was largely devoted to its morphology and cultivation. He has given it the name Euglenamorpha hegneri. (Wenrich, 1923).

studied were of approximately the same age at the time they were examined they differed greatly in size. Those in the pond without algæ being on the average approximately twice as long in both

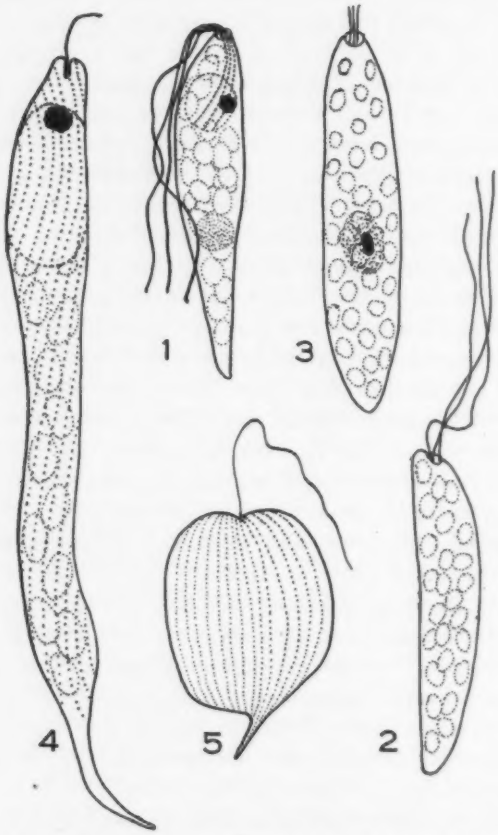

FIG. 1. A living specimen of a euglenoid of Species A showing characteristic shape, three flagella, reservoir, stigma, chromatophores and nucleus. × 1600.

FIG. 2. Species A as seen when stained with iodine. × 1600.

FIG. 3. Species A fixed in Schaudinn's solution and stained with iron-hæmotoxylin. × 1600.

FIG. 4. A living specimen of Species B showing flagellum, reservoir, stigma, and chromatophores. × 1600.

FIG. 5. A living specimen of *Phacus* found in the intestine of *Rana pipiens* tadpoles. × 780.

body and tail and in total length as the others. Every one of the "normal" tadpoles was larger than any one of the "dwarfs."

2. *Length of Intestine.* (Table I.) A high correlation between body size and length of the intestine was found. In almost every case the larger the body the longer was the intestine. The average length of the intestine of tadpoles from the alga pond was less than half that of the other tadpoles.

TABLE I.

MEASUREMENTS OF BODY, TAIL, INTESTINE AND RECTUM, IN MILLIMETERS, OF TEN SPECIMENS EACH OF "DWARF" AND "NORMAL" TADPOLES OF *Rana pipiens.*

	Length of Body.		Length of Tail.		Total Length.		Length of Intestine.		Length of Rectum.	
	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.
10 "Normals"	10–15	13.4	17–24	20.8	27–39	34.2	75–165	127.3	5–20	12.1
10 "Dwarfs"	6–11	8.1	8–15	11.2	15–25	19.3	30–92	58.3	3–10	5.7

3. *Length of Rectum.* (Table I.) The rectum of the tadpole is coiled in such a way that exact measurements are difficult to make. Those in the table are only approximate, but they show that in the "dwarfs" the rectum was only about half as long as in the "normal" tadpoles.

4. *External Evidences of Metamorphosis.*—All of the "normal" tadpoles exhibited rudiments of hind limbs. These measured from 2.5 to 0.5 mm. in length. Only one of the "dwarfs" possessed rudiments of legs and these were only 0.25 mm. long. The former had therefore progressed further in metamorphosis than the "dwarfs."

5. *Internal Evidences of Metamorphosis.*—Shortening of the intestine occurs during the metamorphosis of tadpoles. In judging this character, however, one must take into account the size of the tadpole. The shorter length of the intestine of the "dwarf" tadpoles is probably due to the small size of the animal rather than to a more advanced stage of metamorphosis. In both groups of tadpoles the rectum was well differentiated.

6. *Character of the Contents of the Intestine.*—The intestines

of the two groups of tadpoles differed in appearance, the difference being due apparently to the presence of algæ in the dwarfs and the absence of algæ in the normals. The intestines of the latter were filled with very fine particles of mud mixed with diatoms and minute organic debris; they were grayish in color, of uniform thickness, and smooth in outline. The intestines of the dwarfs, on the other hand, contained large pieces of algæ which appeared to divide the contents into separate masses, giving the entire intestine a patchy appearance; they were slightly tinged with green, and had an irregular outline due to the irregular distribution of the contents. The organisms noted in the intestines of both sets of tadpoles included a very few normal inhabitants of the rectum—*Opalina*, *Trichomonas*, and *Hexamitus;* at least 60 per cent. of both sets were inhabited by *Giardia agilis;* euglenoids were found in four of the dwarfs and one of the normals; and filamentous algae were present in considerable abundance in all of the dwarfs but in only one of the normals.

7. *Character of the Contents of the Rectum.*—The contents of the rectum of the normal tadpoles gave this part of the alimentary canal a grayish appearance, but in the dwarfs the euglenoids were so abundant that a distinct greenish color was produced. It was difficult to count accurately the number of specimens of the various organisms present. The method employed was to mix thoroughly on a slide the entire rectal contents in one drop of normal saline solution; spread this out under an 18 mm. square cover glass and count the number of organisms in ten separate fields in different regions of the slide using a 4 mm. objective and a 4 × ocular. Averages for each tadpole were then computed. Table II. gives the range and the average numbers of five different organisms. *Endamœbæ* and other forms were encountered but were not recorded. *Opalina*, *Trichomonas*, and *Hexamitus* were present in every tadpole and no significant differences in number were noted. The most conspicuous difference in the two groups of tadpoles was the presence of large numbers of euglenoids and of considerable amounts of filamentous green algæ in every one of the dwarfs and the almost complete absence of these in the normals. One or several euglenoids were found in the rectal contents of six of the normals after long searching and a little algæ was seen in one of them.

TABLE II.

COMPARATIVE NUMBERS OF SPECIMENS OF CERTAIN ORGANISMS IN THE RECTUM OF TEN SPECIMENS EACH OF "NORMAL" AND "DWARF" TADPOLES OF *Rana pipiens*.

The numbers were obtained by counting those in ten fields with a magnification of 520 diameters.

	Opalina.		*Trichomonas*.		*Hexamitus*.		Euglenoid Species A.		Filamentous Algæ.	
	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Present.	Abundant.
10 "Normals"	0.2–0.5	0.44	8–32	15.7	15–80	35.7	[1]		1	0
10 "Dwarfs"	0.1–2.0	0.67	3–48	13.6	2–40	23.6	4–60	16.0	8	2

The euglenoids were thus almost entirely restricted to the rectum of the dwarf tadpoles. They seemed to be in excellent condition; the pigment spot was bright red; the chlorophyll bodies were a brilliant green; and swimming activities and metabolic changes were apparently normal.

8. *Conclusions*. This comparative study of 10 specimens each from these two sets of tadpoles leads to the following conclusions:

(*a*) The euglenoid, Species A, is a constant inhabitant of the rectum of the tadpoles of *Rana pipiens* obtained from an alga pond at Ithaca, N. Y., and a rare inhabitant of the rectum of tadpoles of the same species from a neighboring alga-less pond. Infection with *Opalina*, *Trichomonas*, and *Hexamitus* was about the same in both sets of tadpoles.

(*b*) Although of approximately the same age the tadpoles containing many euglenoids were only about one half the size of the other set; and were less advanced in metamorphosis. The presence of these euglenoids may have been a factor retarding growth and metamorphosis.

II. *Effects of Starving the Host on the Persistence of Euglenoids of Species A in Tadpoles of Rana pipiens*.—After a comparison was made between tadpoles of *Rana pipiens* one set of which contained euglenoids in abundance and the other set few or none, it was decided to keep infected tadpoles in the laboratory and examine them at intervals to see if the infection persisted for any

[1] One or several specimens were present in 6 of the 10 tadpoles.

considerable period and if any changes in number, stage in life history, color, etc., would take place. Table III. gives the dates

TABLE III.

NUMBER AND DISTRIBUTION OF EUGLENOIDS OF SPECIES A IN TADPOLES OF *Rana pipiens* COLLECTED JUNE 12 AND KEPT IN LABORATORY WITHOUT FOOD UNTIL DATE OF EXAMINATION.

For method of counting see text.

Date Examined.	Number of Days in Laboratory without Food.	Average Number per Field in Rectum.	Average Number per Field in Intestine.
June 17.....	5	Abundant	Many
June 18.....	6	8	24
June 19.....	7	10	3
June 20.....	8	23	Very few
June 21.....	9	37	Very few
June 22.....	10	19	Many
June 24.....	12	22	Many
June 25.....	13	Many	Many
July 4.....	22	Very few	Many
July 7.....	25	30	Very few

and results of examinations. One tadpole was used each day, the last one being examined on the twenty-fifth day. During this entire period the euglenoids persisted in the digestive tract, in numbers at least as great as in tadpoles examined on the date of collection. My method of counting (see p. 88) was not very accurate, but it seemed to me that the number of euglenoids was more numerous in tadpoles studied on later dates than at first. In the meantime the other protozoa common in the rectum of these tadpoles decreased markedly or disappeared entirely. No encystment was noted in any of the specimens and only one euglenoid was seen in division.

The euglenoids retained their normal free-swimming shape throughout the entire experiment, and were very active, swimming about by means of their flagella or undergoing rapid metabolic movements. No appreciable decrease was noted in the intensity of the green color nor in that of the eye-spot. This was probably due to the transparency of the ventral body-wall which allowed light rays to enter. A few days after the tadpoles were brought into the laboratory the rectum and intestine became almost free from food material and their contents could easily be seen through their walls. It was found that the euglenoids

were usually most abundant in the rectum (see Table III.), almost as numerous in the first 10 mm. of intestine adjacent to the rectum, and fewer in number throughout the rest of the intestine. They were not, as a rule, distributed throughout the intestinal and rectal contents, but could be seen swimming about between these contents and the wall. Often they occurred in large groups thus giving a patchy green color to the digestive tract that could be discerned with the naked eye.

That these euglenoids also persist in tadpoles in nature was proved by the examination of three specimens collected from the same pond on June 24, *i.e.*, 12 days after the first lot were taken. These specimens all contained numerous euglenoids in both rectum and intestine; some of the euglenoids seemed to have become paler in color. The larval period of *Rana pipiens* is from 60 to 80 days but neither the tadpoles collected on June 12 and kept in the laboratory until July 7 nor those collected on June 24 and kept in the laboratory until July 9 increased in size nor advanced in development during this time, although they were about 75 days old and should have been undergoing metamorphosis. The presence of euglenoids may have been a factor in this retardation of growth and development.

III. *Infection of Rana pipiens Tadpoles with Food Containing Species A.*—Tadpoles of *Rana pipiens* in which there were a very few specimens of Species A were collected on June 24, 1922, when about 9 weeks old. On the following day 5 of these were fed on the recta from ten tadpoles of the same species in which there was an abundance of Species A. Previous examination of tadpoles from the same lot as those from which these recta were obtained gave an average number of sixteen euglenoids per field (see Table II., "dwarfs"). The 5 experimental tadpoles immediately began to devour the recta and all of the latter had been eaten by the following day and no specimens of Species A could be found in the dish. Uninfected (normal) and infected tadpoles were examined at intervals of one, three, five, nine, and twelve days. The results obtained are given in Table IV. It is evident from the increase from 0.6 per field to 4.4 per field that Species A has increased in the experimentally fed tadpoles and that this increase is due to the ingestion of specimens contained in the recta used for feeding purposes. Since these specimens were

TABLE IV.

COMPARATIVE NUMBERS OF EUGLENOIDS OF SPECIES A IN THE RECTUM OF TADPOLES OF *Rana pipiens* USED AS FOOD AND IN CONTROL AND EXPERIMENTALLY FED TADPOLES.

Character of Tadpoles.	Number of Specimens.	Numbers per Field of Species A in Rectum.	
		Range.	Average.
Dissected for feeding	10	4–60	16.0
Controls	5	0.2–0.9	0.6
Experimentally fed	5	2–7	4.4

probably all in the free-swimming stage it seems certain that infection of *Rana pipiens* tadpoles can be brought about by the ingestion of active trophozoites. Such a method of infection might occur in nature since tadpoles will feed upon the dead bodies of other tadpoles, but this is probably not the usual method since 100 per cent. of infection has been observed in entire schools of young tadpoles, and, of course, there must be a resistant stage for maintaining the race through the winter and for infecting the first tadpoles in the spring.

IV. *Can Euglenoids of Species A be Cultivated Outside of the Body of ths Tadpole?*—The data presented above indicate that the euglenoids of Species A are regular inhabitants of the digestive tract of *Rana pipiens* tadpoles. The questions suggested by these results are; (1) are these euglenoids restricted to this habitat or can they also maintain a free-living existence; and (2) can other euglenoids known to be free-living be colonized in the rectum and intestine of tadpoles of this species. Two methods of answering these questions were employed: (1) an attempt was made to cultivate Species A outside of the body of the tadpole, and (2) tadpoles were fed on freeliving euglenoids and their digestive tract examined on subsequent days.

Euglenoids of Species A remained alive and active for at least 48 hours inside of the digestive tract that had been dissected out of tadpoles and kept in a small dish in water. Specimens also remained alive for 72 hours in material from the rectum and intestine under a sealed cover glass. Specimens that were dissected

out of the digestive tract and placed in culture dishes did not live and multiply. This does not prove that they cannot maintain themselves outside of the digestive tract of the tadpole but indicates that they probably are restricted to an entozoic existence.

V. *Can Tadpoles of Rana pipiens be Infected with Free-living Euglenoids?*—It has been shown above that Species A can be transferred from one tadpole to another with the food, therefore if free-living species can live successfully in the digestive tract of these tadpoles it should be possible to bring about infection by including them with the food. On June 24, twenty-five tadpoles of *Rana pipiens* about nine weeks old were placed in a culture containing millions of small free-living euglenoids of a species possessing 2 short flagella, obtained from a large tub at the fish hatchery. In 18 hours these tadpoles had eaten every euglenoid in their medium. At this time five of these tadpoles and an equal number of controls were examined. The rectum of the experimental tadpoles was of a deep green color and the intestine also. Not a single specimen of the free-living euglenoids, however, could be found in any of these five tadpoles. The greenish color was due to minute chlorophyll bodies from 3 μ to 8 μ in diameter. These were the chromatophores of the disintegrated euglenoids. Chlorophyll bodies of this type were entirely absent from the five control tadpoles examined at the same time. In both experimental and control tadpoles there were present a few euglenoids of Species A and a few of a species to be described later as Species B. Experimental and control tadpoles were examined on the second day (5 specimens), fourth day (2), and eighth day (1). No euglenoids of the free-living type were discovered. The chlorophyll bodies gradually decreased in number. The conclusion reached is that an essential difference exists between euglenoids of Species A and those of this free-living type, the former being able to withstand the digestive juices of the host and to maintain themselves within the digestive tract, whereas this free-living species is unable to live in the same environment being killed and digested by the tadpole.

A second experiment was carried on at Baltimore during the month of September. Five tadpoles of the green frog that contained very few euglenoids were placed in a small amount of water in which there were thousands of a large reddish-colored

euglenoid. One tadpole that was examined the following day contained many living euglenoids most of which were rounded and quiescent but a few of which were extended though sluggish. Besides these euglenoids there were large numbers of euglenoid chromatophores present proving that many specimens had been broken down within the digestive tract. The feces of the remaining four experimentally fed tadpoles were found to contain living euglenoids and these in the course of the next two weeks must have passed through the digestive tract of these tadpoles and been reingested again and again. One tadpole was killed and examined four days after feeding, another 13 days after feeding and the last two 20 days after feeding. The first two of these contained living euglenoids in both intestine and rectum, but the other two, which were prevented from reingesting their feces for a period of 7 days were entirely free from euglenoids. These results show that the free-living euglenoids used in this experiment had a higher degree of resistance to digestion within the tadpole than those employed in the first experiment but were unable to maintain themselves for a period of 20 days within the digestive tract.

A third experiment of a similar type was carried out with euglenoids obtained from the bladders of the bladderwort, *Utricularia*. I am indebted to my colleague, Mr. Bruce D. Reynolds, for calling my attention to this form and obtaining material for me. Plants obtained from a pond on the campus of the Johns Hopkins University were well supplied with these euglenoids although the surrounding water was entirely free from them. The numbers of euglenoids counted in ten bladders ranged from 8 to 510 in each, with an average of 215. Euglenoids were dissected out of 90 bladders and placed in a dish of water with three tadpoles of the green frog. The total number of euglenoids in this dish was thus about 20,000. All of these had disappeared from the water by the following day and none could be found in the fecal material in the dish. One tadpole was examined after two days and the other two after three days. No euglenoids were found in any of them. This type of euglenoid, therefore, is unable to withstand the digestive juices of the tadpole, although resistant to the secretions within the bladder of the *Utricularia* plant.

VI. *Euglenoids of Species A in Toad Tadpoles.*—A large number of toad tadpoles were collected from one of the large ponds at the fish hatchery on June 12, 1922. Their average measurements were: total length, 20 mm.; body, 8 mm.; tail, 12 mm.; hind legs, 3 mm.; intestine, 47 mm.; rectum, 9 mm. They were kept in the laboratory in a flat dish without change of water but with the addition of fresh water from time to time to compensate for evaporation. Some of the tadpoles developed fore legs and acquired the characteristics of the young toad within a few days; these apparently had reached a stage when no more food was necessary to bring this about. In most of them, however, growth and metamorphosis were inhibited by the condition of starvation to which they were subjected. Specimens were examined at intervals with the results presented in Table V. The following

TABLE V.

NUMBERS OF EUGLENOIDS OF SPECIES A IN THE RECTUM OF TOAD TADPOLES COLLECTED ON JUNE 12, 1922, AND KEPT WITHOUT FOOD.

For method of counting see text.

Date Examined.	Number Examined.	Number of Days in Laboratory without Food.	Range in Number of Specimens per Field.	Average Number of Specimens per Field.
June 28.....	10	16	1–12	5.9
July 11.....	3	29	4–6	5
July 13.....	2	31	1–8	4.5

observations seem worthy of mention. (1) The incidence of infection with Species A was 100 per cent. (2) No encysted or dividing specimens were encountered. (3) Most of the euglenoids were swimming freely or undergoing metabolic movements; a few were spherical or pear-shaped. (4) The number of specimens was not diminished or increased by the starvation of the host. (5) Evidently Species A is a "normal" inhabitant of the rectum of toad tadpoles in this locality. (6) A decided decrease in the intensity of the green color of the chromatophores was noticeable in almost all specimens; some were pale green and others were almost colorless. This condition probably resulted from lack of light and may be contrasted with that observed in the case of specimens from starved tadpoles of *Rana pipiens*.

In the latter the abdominal body wall allows the light to penetrate to the intestine more easily than in the toad tadpole, which is characterized by the presence of dense black pigment. The nutrition of the euglenoids in the toad tadpoles thus becomes almost entirely by absorption whereas in the tadpoles of *Rana pipiens* it is still partly holophytic. It is interesting to note that Species A is sensitive to light, congregating on the side of the slide toward a north window and moving from one side of the slide to the other, a distance of 16 mm. in about 20 minutes, when placed opposite this window.

VII. *Infection of Toad Tadpoles with Species A by Association with Infected Tadpoles of Rana pipiens.*—Evidence was presented above that tadpoles of *Rana pipiens* can be infected with euglenoids of Species A by feeding them food containing active trophozoites, but this probably is not the method of infection in nature. Inasmuch as all tadpoles in certain ponds were found to be infected and none or a very few in other ponds, infection by association was suggested. To test this method the following experiment was carried out. A large number of toad tadpoles were collected on July 10. Five of these were examined on July 23 and found to be uninfected. Seven of the remaining tadpoles were then placed in a finger bowl with 7 tadpoles of *Rana pipiens* taken from a lot that were all infected. They were kept together for five days. Three of the toad tadpoles were still alive, three had recently died, and one had died previously and been removed. Each of the three living toad tadpoles, on examination, was found to contain large numbers of Species A in the rectum and a few in the intestine. No specimens were found in one of the dead tadpoles but a few were present in the other two dead tadpoles. At this time ten more toad tadpoles from the control lot were examined; eight of these were uninfected and one specimen of Species A was found after careful search in the rectum of each of the other two. These results prove that uninfected tadpoles may become infected by associating closely with infected tadpoles. The obvious way in which this is brought about is the escape of specimens from the rectum of infected tadpoles into the water and their ingestion by the uninfected tadpoles. This probably occurs in nature only when the tadpoles are closely associated. Toad tadpoles are very gregarious and might easily

infect one another even in a large pond. Tadpoles of other species are much less accustomed to congregate in numbers and hence would not transfer their infection unless confined to a small body of water.

VIII. *Species A in Tadpoles of the Green Frog, Rana clamitans.*—Euglenoids of Species "A" were encountered in very small numbers in tadpoles of the green frog. For example, several specimens were found in the rectum of each of four tadpoles that were collected on June 14 and kept in the laboratory without food until June 26. That these euglenoids may be taken in with the food of the tadpole and localized in the rectum is evident from the following experiment. One tadpole of the green frog collected on June 14 was starved until June 28 and then fed the rectal contents of three toad tadpoles containing euglenoids. Two days later a considerable number (two per field) of Species A were found in the recutm of this specimen—a much greater number than had been found in any of the several hundred other green frog tadpoles previously examined. Furthermore most of these specimens were pale in color like those in the toad tadpoles at this time.

IX. *Specificity of Euglenoids of Species A.*—It seems probable from the observations and experiments recorded above that euglenoids of Species A are "normal" inhabitants of the recutm and intestine of tadpoles of the frogs and toads that inhabit freshwater ponds. Those living in different hosts may be specifically distinct but no evidence of this was obtained. Attempts to infect adult salamanders proved negative. Ten infected toad tadpoles were fed to a specimen of *Diemyctylus viridescens* on July 1. Four days later no traces of euglenoids could be found in the digestive tract of this specimen, indicating that this salamander cannot be infected with its food and that these euglenoids are probably unable to live in adults of this species. Another specimen of this species of salamander was placed in a dish containing millions of free-living euglenoids. Four days later, the intestine of this animal contained green chlorophyll masses, but no recognizable euglenoids.

X. *Euglenoids of Species B.*—A second type of euglenoid was noted in the rectum and intestine of tadpoles of *Rana pipiens* and *R. clamitans*. Euglenoids of this species, which may be

referred to here as Species B, probably were present in all of many *Rana pipiens* tadpoles collected when about 9 weeks old. They were recorded incidentally in 32 of them, although very little attention was devoted to them at the time. They were noted also in five large tadpoles of the green frog but were probably also of general occurrence in this species. Species B is a cylindrical, elongate species with a blunt anterior end and a posterior end terminated either by a short subacute tip or by a longer process which is slender, pointed at the tip and slightly curved. No free swimming forms were seen but most of the specimens were undergoing slow metabolic movements. Specimens ranged from 99 μ to 128 μ in length and from 9 μ to 16 μ in breadth, with an average length of 110 μ and average breadth of 12 μ. A single short flagellum that moved too slowly to disturb the particles in the surrounding medium was observed in several specimens. Numerous disciform green chromatophores and a large reservoir and red stigma are present. The periplast is spirally striated and strongly punctated in some specimens. In many respects it resembles *Euglena spirogyra* Ehren. Fig.4 is a camera lucida sketch of a living specimen magnified 1600 diameters.

Euglenoids of Species B were never numerous in any tadpole. They were more often observed in the intestine than in the rectum. Their ability to live in these environments and their frequent occurrence indicate that they are normal inhabitants of the digestive tract of tadpoles. An attempt was made to increase their numbers by feeding the rectum and intestine of infected tadpoles to other infected tadpoles. Thus the digestive tracts of 22 tadpoles were fed to four tadpoles of *Rana pipiens* on July 4. Not all of this food was eaten and after 24 hours living active specimens of both Species A and Species B were observed within the walls of the intestines and recta offered as food. Specimens were still alive and active after 48 hours. One tadpole was killed and examined after an interval of three days (July 7) and the remaining three after six days (July 10) but no increase in the numbers of Species B could be ascertained with certainty. I believe there was an increase but the actual numbers were too small to determine the point definitely.

XI. *Species of the Genus Phacus.*—In a few of the tadpoles of

Rana pipiens a species of *Phacus* was noted that resembled in size and shape, *Phacus pleuronectes*. They were about 42 μ long and 32 μ broad, oval in shape with an uncinate posterior spike about 6.5 μ long, a longitudinal groove extending anteriorly from the posterior end, a longitudinally striated periplast, and a large number of green chromatophores, as indicated in Fig. 5. These organisms remained alive and active for 48 hours in intestinal contents that were sealed under a cover glass with vaseline.

XII. *The Effects of the Presence of Euglenoids on Other Intestinal Protozoa.*—No apparent effects on the number and distribution of other intestinal protozoa were noted due to the presence of Euglenoidina. There was, however, a change in the color of many of the opalinas. As a rule *Opalina* is almost transparent when studied with a compound microscope but whenever euglenoids were abundant many of them took on a greenish yellow tinge. This was true in the tadpoles of both *Rana pipiens* and the toad and was very noticeable in the tadpoles that were fed on free living euglenoids. Evidently nutrition in *Opalina* in the presence of chlorophyll bearing bodies is a process whereby some of this coloring matter is taken in thus causing a greenish tinge.

XIII. *The Parasitic Habit among the Euglenoidina.*—Only a few of the Euglenoidina have been described as parasitic in habit. Prof. L. B. Walton mentions two species of the genus *Astasia* in his monograph on the Euglenoidina (Walton, 1915) and has called my attention (in litt.) to other members of the order described by Alexeieff (1912) and Nieschultz (1922). *Astasia captiva* is endoparasitic in *Catenula lemnae*, and *A. mobilis* in the egg sacs and digestive tract of *Cyclops*—a condition that has led to the suggestion by Alexeieff that parasitic Sporozoa may have originated in this way. Nieschultz describes a species of *Astasia* from the digestive tract of a fresh-water nematode, *Nilopus gracilis*, but does not give it a specific name. Haswell (1907) reported the occurrence of a euglenoid inside the cells of a rhabdocœle Turbellarian. Alexeieff reports the discovery of small, living, active euglenoids, *Euglena* sp. and *Phacus longicauda*, in frog tadpoles and states that Brumpt also showed him specimens containing hundreds of *Euglenæ*. The green color and stigma of these were as bright as in free-living specimens. This, accord-

ing to Alexeieff, is a case of accidental parasitism which is a stage in the evolution of parasitism among the Euglenoidina. He accounts for the presence of these euglenoids by the mode of nutrition of the tadpoles, which engulf large quantities of debris of all sorts resulting in a thick, compact mass in the intestine. Organisms in the midst of such a mass might easily escape the action of the digestive juices, especially since these are greatly diluted, and as a result become gradually acclimated and finally facultative parasites.

XIV. *Summary and Conclusions.*—(1) Three species of Euglenoidina are described from the intestine and rectum of frog and toad tadpoles; all three species possess green chromatophores and bright red stigmas.

(2) A comparative study of two sets of tadpoles of *Rana pipiens* from adjoining ponds, one set much retarded in growth and heavily infected with Species A and the other of normal growth but lightly infected or not at all, indicates that the dwarfing of the former may have been due to the presence of euglenoids.

(3) Tadpoles of *Rana pipiens* infected with Species A were kept in the laboratory without food for 25 days and specimens examined at intervals. The infection persisted throughout this period without any marked decrease in the brightness of the green color of the euglenoids. Only one case of division was noted, and no cysts were found, the euglenoids remaining as trophozoites, free swimming and actively metabolic, throughout the period. The retention of the green color may have been due to the transmission of light through the almost transparent abdominal and intestinal wall. The rectum is the usual habitat of this species but the intestine is often invaded especially the first 10 mm. just anterior to the rectum. They are not mixed with the intestinal and rectal contents but move about between this mass and the containing walls. Infection was found to persist in tadpoles collected from time to time from the pond.

(4) Tadpoles of *Rana pipiens* containing very few specimens of Species A were fed on the intestines and recta of highly infected tadpoles of the same species. A great increase in the number of Species A in the experimentally fed tadpoles proves that infection with this species can be brought about by the ingestion of active trophozoites with the food. This, however,

is probably not the usual method of infection in nature, since a resistant over-wintering form of Species A probably exists by means of which the new broods of tadpoles are infected in the spring.

(5) Attempts to cultivate Species A outside of the tadpole failed and it seems probable that trophozoites are incapable of living and reproducing themselves in water outside of the host. Specimens were kept for several days in intestines and recta that had been dissected out and placed in water and also in rectal contents sealed under a cover glass. The latter proved to be sensitive to light congregating on the side of the slide toward a north window and moving from one side of the slide to the other, a distance of 16 mm., in about 20 minutes, when placed opposite this window.

(6) An attempt was made to infect tadpoles of *Rana pipiens* with three species of free-living euglenoids. The euglenoids were all ingested but none became colonized in the digestive tract. One of these euglenoids was taken from the bladders of *Utricularia* in which they were able to maintain themselves in spite of the secretions present there. The species that inhabit the intestine and rectum of the tadpoles therefore possess a resistance to digestive juices not present in free-living forms.

(7) All of a large group of tadpoles of the toad, *Bufo lentiginosus americanus*, were found to be infected with Species A, but another group of tadpoles of this species from another pond were not infected. Specimens of infected tadpoles were kept in the laboratory without food for 31 days and examined at intervals. The euglenoids persisted throughout this period. No apparent increase in numbers was noted and no division stages nor cysts were observed. There was no decrease in size. The organisms were free-swimming and actively metabolic. The green chromatophores, however, gradually became paler in color until the specimens were almost transparent. This loss of the green color was probably due to the failure of sufficient light to penetrate the deeply pigmented abdominal wall of the toad tadpole.

(8) Euglenoids of Species A were also found in the intestine and rectum of tadpoles of the green frog, *Rana clamitans*. They were not as numerous as in tadpoles of *Rana pipiens* or of the toad. One tadpole that was fed on the intestines and recta of three infected toad tadpoles became more highly infected, thus proving

that active trophozoites from the latter can be transferred to tadpoles of the green frog with their food.

(9) It is evident that euglenoids of Species A are regular inhabitants of the intestine and rectum of three species of tadpoles, *Rana pipiens*, *R. clamitans*, and *Bufo lentiginosus americanus*, and that they can be transferred from tadpoles of one species to those of another with the food. No specific differences were noted in specimens from different species of tadpoles.

(10) Euglenoids of Species B were present in tadpoles of *Rana pipiens* and *R. clamitans*, but were never very numerous. The intestine seemed to be more highly infected than the rectum. The specimens observed were as green as free-living species and contained brightly colored stigmas. Most of them appeared to be without flagella and either remained stationary except for metabolic movements or squirmed slowly from place to place. Attempts to increase the number present in one tadpole by feeding it infected intestines and recta of other tadpoles were not definitely successful.

(11) A species of *Phacus* resembling *P. pleuronectes* was observed in a few tadpoles of *Rana pipiens*.

(12) The presence of euglenoids had no apparent effect on other protozoan inhabitants of the digestive tract except in the case of certain opalinids which became yellowish green in color.

The advantages of the group of organisms dealt with in this paper as material for a study of the evolution of parasitism is obvious and the writer expects to continue work on the group with this object in view.

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